THE DEVELOPMENT OF SOME EXOGENOUS SPECIES OF AGARICS

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In 1914, while looking for agaric material suitable for developmental studies, abundant young stages of several exogenous forms were found. At that time Professor Atkinson pointed out the need of a thoroughly modern investigation of these forms, both on account of the incompleteness of the early work and because the method of gill development in the endogenous forms was at that time receiving considerable attention. It seemed important to determine if the method of gill formation of species, differing in the presence or absence of a universal veil but otherwise rather closely related, would show any very radical differences in the method of the formation of their gill salients.

Until the work of Blizzard (9) in 1916, no study of development of exogenous forms of agarics had been made with our modern methods of technique. A considerable number of exogenous as well as endogenous forms[1] were early studied by Hoffmann (17, 18, 19) and among them one species of *Entoloma* (*E. sericeum* Bull.). Although his work was of necessity somewhat limited, he came to the correct conclusion with regard to the differentiation of the hymenophore primordium in the groove formed between the stem and pileus fundaments, which had been formed by the epinastic growth of the pileus hyphae. He also noted the formation of an even palisade layer before the appearance of the gill ridges.

Four exogenous forms, among them being one *Mycena* (*M. vulgaris*), were studied by DeBary, whose final conclusion (13, 14) with regard to the formation of an even palisade layer, preceding the development of the gill ridges, agreed with that of Hoffmann (17, 18, 19). In addition he emphasized the order of their development in a centrifugal manner from the stem to the margin of the pileus.

In 1889 a great number of forms were studied by Fayod, but owing

[1] For a complete list of the early forms studied see Atkinson, G. F., Origin and development of the lamellae in *Coprinus*. Bot. Gaz. 61: 89. Footnote 1, 1916.

to his rather indefinite limitations of the "couche piléogène" and the "primordial cuticle," and also to the fact that he considered all the forms to arise endogenously, there is considerable need for reinvestigating these forms.

The excellent work of Blizzard (9) in 1917 has confirmed, in the case of certain species of *Omphalia*, *Clitopilus* and *Clitocybe*, the final conclusion of DeBary and Hoffmann. This present paper is offered as a further contribution to our knowledge of the exogenous forms.

MYCENA SUBALCALINA Atkinson.*

(Figs. 1–24)

One of the autumnal species of fungi, which is found frequently in great abundance in the woods about Ithaca, N. Y., is *Mycena subalcalina*. It is a small mushroom, rarely exceeding 2 cm. in the diameter of its pileus. On account, however, of the profusion in which it grows on decayed stumps and the ease with which it may be collected free from soil particles, it lends itself very readily to morphological study.

Collection and Preparation of Material.—The material for this study was collected at two different times, once on November 7, 1914, from the marsh at McLean, N. Y., and again on January 20, 1915, from the inside of a hollow trunk in the Beech Woods by Six Mile Creek near Ithaca, N. Y. At this time the ground was covered with snow and the fruit bodies, which were exposed on the surface, were covered with a coating of ice, apparently interrupted in their development by the coming on of winter. Some of these latter, together with others from the interior which were not frozen, were immediately fixed in medium chromo-acetic acid. All of the material proved

* **Mycena subalcalina** Atkinson, n. sp. Caespitosa vel subcaespitosa, 3–7 cm. alta; pileo convexo dein expanso, avellaneo-brunneo vel castaneo-brunneo, 1–2 cm. lato, leniter striato, lento, humecto sed non viscido; lamellis albis demum subsordidis, angustatis, subdistantibus, arcuatis, adnatis, dente decurrentibus; cystidiis nullis vel raris, ad aciem lamellarum clavatis, non emergentibus, frequenter mucronatis; sporis quaternis, levibus, subellipsoideis vel ovalibus, minutissimis, $3–6 \times 2–2.5\,\mu$; stipite sursum albo vel pallido, deorsum luteo-rufescenti, ad basem atrobrunneo et strigoso, apice leniter pruinoso, 2–3 mm. crasso.

On decaying wood in the vicinity of Ithaca, N. Y. The plants are tough and pliant when fresh, with an alcaline or nitrous odor, though sometimes faint.

satisfactory, the freezing apparently not affecting the buttons. After washing and dehydrating they were cleared in cedar oil and embedded in 52° paraffine. Sections were cut from 5–7 microns in diameter and stained with fuchsin.

Early Stages in the Development of the Fruit Body.—It was possible to obtain in this manner fruit bodies in which no differentiation had taken place. A longitudinal section of a young button, about 2 mm. in diameter, is shown in figure 1, attached to the base of a more developed plant. It consists of homogeneous tissue, composed of small, densely interwoven hyphae, about 1 μ in diameter. The external hyphae are somewhat larger (1.5 μ) and take the stain more readily. Later the young fruit body becomes somewhat flask-shaped, as shown by the section of the larger plant in figure 1. The interwoven hyphae assume a generally longitudinal direction throughout the center of the fruit body, but all over the surface they already exhibit a strong inclination to turn outward. At the apex this epinastic tendency has resulted in a differentiation into two regions, the pileus and stem primordia, separated by an annular constriction or furrow. The tissues of both regions, however, are still homogeneous, that of the upper, in fact, being made up of the extension of the hyphae of the lower.

Differentiation of the Primordium of the Hymenophore.—As the basidiocarp increases in size, the differentiation between the stem and pileus fundaments becomes more marked (fig. 2). As yet there is no distinguishable hymenophore fundament, although a slightly increased staining capacity of the hyphae in the furrow suggests that a rapid growth is taking place at this point. The elements are still similar to those which are characteristic of the margin of the pileus, broad (1.6–3.5 μ in diam.), and blunt at the ends (fig. 19). Very soon the typical hymenophore elements appear. They are slender and sharply pointed at first, as they are in many of the endogenous forms. They branch off very profusely from the subadjacent tissue of the pileus and are stained more deeply (figs. 3, 4, 5, 22).

Development of the Palisade Layer.—The dense crowding of these elements causes them to become organized very soon into a palisade layer. In figure 22, an enlargement of figure 5, we may observe that the under surface of this zone of very active growth is still somewhat irregular, due to the varying lengths of the narrow-pointed hyphae. Later they become more even and blunt and the fruit body presents

the appearance of figures 6, 17, 23. Compared with the thickness of the elements, this palisade layer is an unusually deep one. The hyphae become more and more crowded as a result of their rapid branching and their slight increase in diameter. For this reason the layer becomes very conspicuous in contrast to the looser, more inactive subhymenial tissue, from which these hyphae have arisen.

Development of the Gills.—Fruit bodies in the stage shown in figures 8–12 show the very earliest evidences of gill formation. We have seen that the palisade layer has become very crowded by the multiplication of its elements. As these continue to be formed, this extra growth produces a tension which must be taken care of in some way. This is accomplished by the growth downward of the subadjacent hyphae in regularly spaced radial rows, beginning at the stem and extending to the margin of the pileus. In figure 8 the palisade layer appears to be slightly decurrent on the stem. It is here that the salients first begin to form. These appear as two irregular folds in figure 9, which is reproduced from a section cut parallel to the median plane at the surface of the stem. As one passes outward, these folds become less marked (fig. 10) and finally disappear altogether. In figure 11, nearer the margin, we find the level palisade layer, while at the very outside of the pileus the irregular palisade primordium is present (fig. 12). The series of sections in figures 13–17, from a more mature plant, shows better-developed salients, while figure 18 represents a section through the margin. These salients are becoming more regular and, as the pileus broadens and the interstitial growth forces the primary salients apart, new secondary ridges make their appearance between the original ones (fig. 21). This method of gill development is then, in the main, the same as that described by Hoffmann (**17**, **18**, **19**) and DeBary (**11**, **12**, **13**, **14**) in exogenous forms and also agrees with species recently studied by Blizzard (**9**). It is also similar to the method of gill origin in endogenous forms of the *Agaricus* type described by the earlier workers and in the modern work of Miss Allen (**1**), Atkinson (**2**, **3**, **4**, **5**, **6**, **7**), Beer (**8**), Douglas (**15**), Sawyer (**20**) and Zeller (**21**), with the exception that in the endogenous forms, the ridges develop underneath in a more or less distinct "gill cavity," while in the exogeneous forms they are exposed from the first to the outside.

Development of the Pileus.—After the appearance of the annular furrow separating the pileus primordium from that of the stem, the

pileus grows very rapidly (fig. 2). Its texture is at first similar to that of the stem, since its hyphae, as mentioned above, are but the continuation of those of the stem which have bent outward under the influence of epinasty. By growth and branching new elements are being continually added, especially at the margin of the pileus. When the primordium of the hymenophore appears, followed by the level palisade layer with its compact row of parallel hyphae, the pressure causes the subhymenophore tissue to become stretched, looser and more open in texture. We noticed in the earliest stage a layer of more deeply staining tissue over the surface of the fruit body (fig. 1). It persists throughout the early stages and appears to be composed of enlarged hyphal cells (in fig. 6, 4 μ in diameter) which have been chemically changed or injured as they have grown through the substratum. They persist for a time in loose tufts, finally disappearing with the maturity of the plant.

Development of the Stem.—As the plant grows, the stem elongates rapidly and at the same time grows in thickness by the interpolation of new elements, which intertwine with each other but which take a general longitudinal direction. On the surface they bend strongly outward (figs. 1–13) instead of uniting to form a compact layer as they do in many forms with a smooth stem. The villous stipe of the young growing plants is due to this circumstance.

Hygrophorus

Collection and Preparation of Material.—Three species have been studied. The material used in the study of *H. miniatus* came from two different collections. Pieces of a decayed stump, containing young fruit bodies, were brought into the laboratory from McGowan's woods, near Ithaca, in October, 1915, and from these the young fruit bodies were chosen. Another collection was made by Mr. Blizzard at Seventh Lake, Adirondack Mts., N. Y., during August, 1916, from which the early stages in the development of the gills were obtained. The specimens of *H. borealis* were collected by Professor Atkinson and the author from rich leaf mold where numerous scattered mature plants were growing, in the sphagnum moor at Malloryville, N. Y., during August, 1914. *H. nitidus* plants were found growing in rich soil by the edge of Eighth Lake, N. Y., in August, 1916. The material of all three species was fixed in medium chromo-acetic

acid, cleared in cedar oil and embedded in 52° paraffine. Sections were cut from 5–7 microns in diameter and stained with basic fuchsin and carbol fuchsin.

HYGROPHORUS MINIATUS Fr.

(Figs. 25–47)

Young Stages.—Fruit bodies up to 1 mm. in length show no differentiation of tissues (fig. 25). The buttons are more or less conical and composed of compactly interwoven hyphae about 1.5 μ in diameter with conspicuous nuclei. A number of the fruit bodies, in about this stage of development, show, distributed throughout the tissue, other hyphae which are considerably larger in diameter and which have a strong affinity for the stain. They are possibly in the process of disintegration or are food-storage hyphae. They are also found in some of the later stages but have completely disappeared by the time that the hymenophore primordium is organized. It was suggested by Professor Atkinson that they probably function as nutritive elements for the other rapidly growing hyphae. The young buttons elongate very rapidly and become flask-shaped with long, pointed necks.

Differentiation of the Fundaments of Pileus, Stem and Hymenophore.—Later the fruit bodies undergo considerable broadening at the apex, leaving a decided groove, which separates the nearly oval pileus fundament from that of the elongate stem (fig. 26). In this latter region the primordium of the hymenophore later appears (fig. 27). The delineation of the pileus and the stem regions is not due to differences in the nature of their tissues but, as in the case of *Mycena subalcalina*, to the growth and rapid multiplication of the hyphae of the pileus fundament, which exhibit a strong tendency to turn outwards and downwards. Certain ones at the top of the stem and on the under side of the pileus show this inclination more strongly than the others and become directed outward in a nearly perpendicular direction to the tissues from which they arise. They are narrower than the elements at the pileus margin, have sharp ends, and become very numerous by successive branching. These hyphae constitute the primordium of the hymenophore (fig. 27).

Development of the Gills.—Differing from the condition found in the majority of forms, developing according to the *Agaricus* type, thus far studied, the appearance of gill salients precedes the formation of a definite, even palisade layer. While the elements of the primordium

continue to increase, the subadjacent tissue next the stem begins to extend downward in radial lines carrying the hymenophore primordium with it (figs. 28, 29 and 44). Gradually the palisade layer becomes more even (figs. 30–34, 47). By centrifugal growth the gill folds progress toward the margin of the pileus, where new primordial tissue is being organized (fig. 34). As in the case of *Mycena subalcalina*, the pressure of growth in the hymenophore region causes a loosening of the subadjacent tissue (figs. 29, 32, 47). More advanced stages in the development of the gills are shown in the fruit bodies represented in figures 35–39 and 40–42. As the gill ridges continue to grow, they become very broad with large spaces between. They finally assume the more or less triangular shape in cross section, which is one of the distinguishing characteristics of the genus *Hygrophorus* (figs. 41, 42). The trama, as can be seen in figure 40 in surface section and in figure 41 in cross section, is similar in character to that of the pileus.

The palisade layer, when finally organized, may be studied in photographs of figures 41, 45, 46. It shows especially well on the older part of the gills nearest the pileus. The ends of the hyphae have become very crowded and blunt, thus bringing about an even surface on the gill. No nuclei are present in the extremities but they are very conspicuous and deeply stained at the bases of the palisade layer cells. The adjacent tissue contains very abundant and deeply stained nuclei and presents much the same appearance as it did in species of *Cortinarius* (15). Just above this is the loose open zone which extends into the gills and into the pileus region between them, subadjacent to the hymenophore, and which gives rise by branching to the hyphal elements which make up the hymenophore. The palisade layer is not as compact (fig. 41), the hyphae have become clavate and the nuclei have migrated into the tips. These hyphal ends are apparently young basidia in the process of forming.

Structure of the Stem and Pileus.—The cap does not separate easily from the stem in mature stages, a fact which is due to the homogeneous character of the tissues of pileus and stem in gymnocarpous forms. It is made up of the continuation of the hyphae of the stem, as we observed in *Mycena subalcalina*. At the surface no blematogen is present and there is practically no change in the character of the tissue. The ends of the hyphae grow somewhat unevenly, so that the surface is at first irregular (fig. 28). Later on the ends become swollen and are cut off by cross walls (fig. 43).

HYGROPHORUS NITIDUS *B* AND *C*

(Figs. 48–66)

Young Stages.—In the earliest stage obtained of this form, a button about 5 mm. in length (fig. 48), differentiation of the pileus and stem regions had already begun to take place. In the photographs, the actively growing region appears by its deeply staining property to be at the tip, where the longitudinal hyphae are now multiplying profusely and growing outward in the formation of the pileus fundament. By the time development has reached the stage shown in figure 49, not only is the pileus primordium well delineated from that of the stem but we also have in the annular furrow between them the primordium of the hymenophore. Even though the whole surface of the fruit body is clothed with a layer of outwardly directed hyphae which take the stain deeply, this layer is differentiated by a still deeper stain and by somewhat smaller hyphae (about 2 μ in diameter as against those 3 or 4 μ at the margin of the pileus). These differences appear more sharply in an older stage (figs. 50, 51, 52). The hyphal ends on the surface appear to be disintegrating. This species is a viscid one and these deliquescing hyphae furnish the slime which covers the plant.

The Palisade Layer.—Although the hymenophore elements become crowded into a palisade-like layer, the surface of the gills never becomes smooth as it does in most of the endogenous forms studied and in the early stages of the two preceding species. Their hyphal ends are variously directed and uneven in length (figs. 53–57). This character is retained even in fairly mature stages (figs. 59–63, 66), whose surface in consequence is always uneven. The layer is, however, a very definite one and is homologous with the more even layers of the other species.

Formation of the Gills.—The first evidence of gill salients appears in the fruit body at a stage represented in figures 53–58, which shows the palisade layer being pushed out into very low undulations. In the next series (figs. 59–63), the gill character shows more clearly and it becomes quite evident that the method of formation is identical with that of *Hygrophorus miniatus*, previously described. The sections were taken from a fruit body which was growing close beside a second one of the same age, the margins of the two at the point of

contact having grown together (fig. 59). The trama of the gills has a very loose mesh which brings into sharp relief the deeply stained, crowded palisade layer, which covers the surface of the gills (fig. 59). In figure 66, an enlargement of the section shown in figure 61, the very uneven character of the palisade layer on the edge of the gills is apparent. The triangular form of the gill in section appears in the nearly mature plant of figures 64 and 65. The tissue of this fruit body is still homogeneous in character, except for the gills and the slime-producing layer over the surface. The portion of the pileus at this time is beginning to take on a hyponastic growth, causing the mushroom to become umbilicate.

Hygrophorus borealis Pk.

(Figs. 67–80)

Early Stages.—The development of this species resembles that of *H. miniatus* and *H. nitidus* so closely that it will not be necessary to enter into its life history with as much detail. The youngest button found was nearly oval in shape and entirely homogeneous in composition (fig. 67). For a time after this the fruit bodies elongate very rapidly (figs. 68, 69), growing chiefly at the apex, as shown by the deeper stain in this region. At length, when they are well up out of the soil, the hyphal elements in the apex multiply and turn outwards in epinastic growth. Thus the pileus is formed (figs. 70 and 71). At practically the same time, the hymenophore primordium becomes organized (figs. 70, 71 and 72) in the annular constriction between the fundaments of pileus and stem. A very strong epinastic growth causes the margin of the pileus not only to bend downward but to inroll. Thus a protection is obtained for the young gill salients as they form. Before their appearance, however, a definite, even palisade layer is formed (figs. 73, 74 and 75).

Development of Gills.—Unfortunately very early stages in the formation of the gills are lacking. Figures 76–80 represent the youngest stage found. It corresponds very nearly to the stage represented in figures 36–39 in *H. miniatus* and shows the progressive maturity of the young ridges, as one passes from the margin (fig. 80) inward to the center (fig. 76). In figure 80 the dark stain represents the hymenophore primordium seen in surface view on the inside of the inrolled margin of the pileus. Figure 81 represents a very nearly mature

plant in radial section. The cut is made between the gills, but a portion of one gill joined to the stem appears in the photograph. The lightly stained area subadjacent to the palisade layer in figures 77–80 represents the layer from which the hymenophore elements have branched. This extends not only into the gills but also between them in the pileus region, subadjacent to the hymenophore. The corresponding region is shown very clearly by Blizzard (9) in *Omphalia chrysophylla* (Plate VII, fig. 28) where certain hyphae give rise by digitate branching to the palisade layer.

Entoloma

Collection and Preparation of Material.—Embedded material of three species was turned over to me by Professor Atkinson, who had collected the young stages of *E. flavifolium* and *E. grayanum* from rich leaf mold in the Michigan Hollow Swamp near Danby, N. Y., in September, 1914. The material of *E. cuspidatum* was gathered during July, 1916, from humus among sphagnum in the woods near Seventh Lake, N. Y. All of the material was fixed in the field in medium chromo-acetic acid. It was cleared in cedar oil and embedded in 52° paraffine. Sections were cut from 5 to 7 μ in diameter. Great difficulty was experienced in staining them. The young stages remained practically unstained in a great variety of the common stains. Finally the method which proved satisfactory for some of the resistant *Cortinarius* (15) species, that of using tannic acid as a mordant followed by the fuchsin stain, was tried with fairly good results. Iron-alum haematoxylin was very satisfactory for older stages.

E. flavifolium Pk.

(Figs. 82–100)

Early Stages.—The youngest button which was found is flask-shaped, about 3 mm. in length and 1 mm. through the widest part at the base (fig. 82). It is very compact in structure, especially in the region of the tip, where the hyphae are densely interlaced. The latter are slender and average about 2 μ in diameter. The many prominent nuclei are an indication that active growth is taking place here. Already this has resulted in the differentiation of an enlarged apex, the pileus primordium, from that of the stem. As one passes

to the base of the fruit body, where growth is less active, the tissue becomes more and more loose, the hyphae broad (6 μ), and almost unstained. Completely covering the young button is a thin, deeply stained layer, made up of large, thick-walled hyphae, in a more or less complete state of disorganization. This is evidently not a protoblem, such as is present in *Agaricus compestris* (3) and probably in certain species of *Cortinarius* (15), but appears to be formed as in the case of *Mycena subalcalina* by a transformation of the tissue on the outside of the fruit body by substances in the substratum, though which it has pushed its way. As the plant grows older, this outer stratum of dead elements is exfoliated.

The Primordia of Pileus, Stipe and Hymenophore.—When the fruit body has reached the stage of figure 83, a sharp constriction has formed below the free end by the branching and epinastic growth of the hyphae. As in the preceding species, this groove marks the division between the primordia of the stem and pileus. The whole fruit body has increased considerably in size, partly on account of the spreading out and loosening of the tissue and partly because of the addition of new elements. On the top of the pileus fundament the ends of the radial fibers break off in small mats or tufts, while just beneath the strongly curved margin and extending for a short distance down the stem is now distinguishable the primordium of the hymenophore (figs. 85, 86). This region, contrary to the condition in the preceding species, does not take the stain readily. It appears on closer examination to be composed of blunt hyphae with a somewhat irregular direction, but showing a marked tendency to turn downward perpendicularly to the pileus or outward from the stem.

The Palisade Layer.—These hymenophore elements increase very rapidly by branching and at the same time become considerably broader, so that a very compact palisade layer results (figs. 85, 86 and 98). It still is very resistant to the stain, but is easily distinguishable from the looser pileus and stem tissues adjacent to it.

The Development of the Gills.—The method of development of the gill salients is precisely the same as described in the preceding species in this paper and in the case of the previously mentioned endogenous forms. Figures 87–90 represent serial sections from the youngest fruit body which shows traces of these folds. They are protected in their development by the strongly inrolled pileus margin, which causes the apparent gill cavity in figures 89 and 90.

As soon as the young ridges begin to form, certain deeply staining elements make their appearance among the unstained ones of the original palisade (figs. 89 and 99). They grow rapidly and soon outstrip the others. Later on (figs. 91–95, 100) the unstained elements become completely lost amongst the deeply stained ones, which appear now to be young basidia. Is it not possible that the earlier unstained hyphae represent sterile paraphyses? A somewhat similar condition in *Cortinarius cinnamomeus* (15, Plate XI, fig. 48) suggests a like interpretation for the thin zone on the outside of the deeply stained hyphae. In the median section of this later series (fig. 91) we see the surface view of one of the primary gills. It is interesting to note that the palisade layer formed on the stem is developing into the decurrent tooth of the gill. Figures 92–95 represent longitudinal sections of the same fruit body. As one progresses outward, secondary salients are now making their appearance between the primary ones. Figure 100 is an enlargement of a portion of figure 94 and shows the young basidia pushing out from the surface of the gill just previous to the development of the sterigmata and spores. In figures 96 and 97 the fruit body is practically mature.

Entoloma grayanum Pk.

(Figs. 101–120)

Early Stages.—The development of *E. grayanum* may be considered somewhat more briefly, inasmuch as it follows very closely the method of development described for *E. flavifolium*. The youngest button (fig. 101) has already become sufficiently well differentiated to make distinguishable the primordial regions of stem and pileus. On the surface there is also a layer of tissue which appears to result from the disorganization of the superficial cells, indicating an early stage in the retreat of pileus development from the surface to the interior of the young fruit body. The hyphae making up the fruit body are very compact and take the stain with the greatest difficulty. As the plant increases in size, its tissues become more open and the irregular primordium of the hymenophore makes its appearance in the groove formed by the arching out of the pileus (figs. 103–105). This very soon becomes organized into the even palisade layer shown in figures 106–110, which becomes very compact and remains inconspicuously stained (fig. 110) as in the former species.

Formation of the Gills.—Very early stages in the formation of the gills were not obtained, but the serial section (figs. 111–115) of an older fruit body, which is developing gill ridges, shows stages corresponding to those passed through in the development of *E. flavifolium* (figs. 87–95). The gills are, however, much more crowded than in that species. Certain elements on the surface of the folds in *E. grayanum* stain very deeply and become very conspicuous (figs. 112, 113, 119, 121). These are immature basidia, each of which contains at this stage a single large nucleus (fig. 121). They are not as numerous as they are in an earlier stage in *E. flavifolium*, where they formed a conspicuous stratum in the palisade layer (figs. 89, 99). It may be due to differences in the staining reactions. Figures 116–120 represent a slightly older stage of development. The gill salients are so crowded that they are developing somewhat irregularly. In figure 116 the decurrent tooth on the stem is noticeable.

Entoloma cuspidatum Pk.

(Figs. 122–139)

Early Stages.—Rhizomorphs of parallel hyphae produce the fruit bodies which are at first nearly homogeneous in their composition (fig. 122). By progressive growth at the apex there is formed a button in the stage of figure 123, which is just beginning to show a differentiation into pileus and stem primordia by the formation of an annular furrow. Later (figs. 124 and 125) the differentiation of these two main regions becomes more marked. The tissue in these fruit bodies was somewhat shrunken in the preparation processes, so that one cannot determine in them with certainty whether the primordium of the hymenophore has as yet developed. It can, however, be definitely ascertained in figures 126 and 127 and 138, where it consists of narrow, crowded, pointed elements, which take the stain readily in contrast to the looser subhymenial tissue. This soon gives place to an even palisade tissue (figs. 128, 129 and 137). Here the hyphae have increased in diameter, are very crowded and, as was noticed in the case of *Hygrophorus miniatus*, are more deeply stained next to the subadjacent tissue.

Further Development of the Fruit Body.—The gills appear as in the case of the two previous species as centrifugally growing ridges,

caused by the crowding of the palisade tissue and the pushing downward of the palisade layer by the elongation of the subadjacent tissue in regularly spaced intervals (figs. 130–134). As these folds develop, their surface becomes again uneven (fig. 139) as it did in case of the *Hygrophorus* forms mentioned above. Figure 135 represents a fruit body which is nearly mature. In figure 136 one may observe the spores from a mature plant. As in all the characteristic species of *Entoloma* the spores are angular. In this species they are very nearly cubical.

Summary

1. The fruit bodies of these exogenous forms come from buttons composed of interwoven hyphae, mainly extending in a longitudinal direction; with the exception of the surface layer, which is sometimes transformed by substances in the substratum through which the plants are growing, the tissue is entirely homogeneous.

2. Differentiation of the pileus and stipe primordia is brought about by growth at the apex of the fruit body. The hyphae multiply by profuse branching and begin to turn outward. This soon results in an enlarged pileus fundament, differentiated from that of the stem by an annular furrow. The tissues of both regions remain homogeneous in character throughout the life of the plant.

3. The fundament of the hymenophore is differentiated in the annular furrow. In the three *Hygrophorus* species, it seems to appear simultaneously with the differentiation of the pileus and stipe primordia, while in those of *Mycena subalcalina* and the three *Entoloma* ones, it develops slightly later. It is characterized by crowded, narrow, usually pointed hyphae and by an irregular surface.

4. Before the formation of gill salients an even palisade layer is usually formed by dense, broader hyphae with blunt ends. In two *Hygrophorus* species, *H. miniatus* and *H. nitidus*, however, the surface does not become even until after the gill ridges have formed.

5. The gills are formed from salients which appear first at the stem and develop in a centrifugal manner to the margin of the pileus. The dense crowding of the elements of the palisade layer results in a strong tension of the tissues, which is finally taken care of by the development of the subadjacent tissue downward into regularly spaced radial ridges. This method is precisely the same as that described by the early workers (11, 12, 13, 14, 17, 18, 19) and by Blizzard (9)

for other exogenous forms. Except for the fact that these gills develop on the exposed under surface of the pileus and not within a gill cavity, their method of origin is the same as that of the endogenous forms of the *Agaricus* type recently studied (**1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **15**, **20**, **21**).

In conclusion I wish to express my sincere appreciation to Professor Atkinson for his unfailing interest and helpfulness in the preparation of this paper.

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DESCRIPTION OF PLATES I–VII

Figures 19, 22, 23, 24, 27, 43, 44, 45, 47, 52, 66, 72, 75, 99, 100, 121, 135, 136, 138 and 139 were taken with a Bausch and Lomb compound microscope, fitted with a Zeiss 4 mm. ocular and a 3 mm. objective. For figs. 46 and 110 a combination of ocular 6 and objective 3, for fig. 82 a combination of a 4 mm. ocular and 16 mm. objective, and for fig. 98 an 18 mm. ocular and 16 mm. objective were used. The other figures were photographed by means of an extension camera and Zeiss lenses, figs. 64 and 65 with a 35 mm. objective, figs. 96 and 97 with a 50 mm. objective, and the others by means of a 16 mm. Spencer Lens Co. photographic objective.

PLATE I

Mycena subalcalina (figs. 1–24)

FIG. 1. × 36 diameters. Two young fruit bodies. The larger, by the epinastic growth of the hyphae at the apex, is becoming differentiated into stem and pileus primordia.

FIG. 2. × 36 diam. A slightly older stage, in which the pileus and stem primordia are well differentiated.

FIGS. 3, 4, 5. × 36 diam. Median and two tangential sections of a fruit body, showing the primordium of the hymenophore.

FIGS. 6 AND 7. × 36 diam. Median and tangential sections of a fruit body with the palisade layer developed. The crowding of the palisade elements causes the subadjacent tissue to become very loose.

FIGS. 8–12. × 36 diam. A series of sections from a fruit body, in which the gill salients are just beginning to form.

FIGS. 13–18. × 36 diam. A slightly older series, showing the development of the gills.

FIG. 19. × 255 diam. Enlargement of the margin of the section of fig. 2.

FIGS. 20 AND 21. × 36 diam. Median and tangential sections from a nearly mature fruit body.

FIG. 22. × 255 diam. Enlargement of the section of fig. 5, showing the hymenophore primordium.

FIG. 23. × 255 diam. Enlargement of the section of fig. 7, showing the palisade layer.

FIG. 24. × 234 diam. Enlargement of the section of fig. 16, showing young gills.

Plate II

Hygrophorus miniatus (figs. 25–47)

Fig. 25. × 30 diam. Young fruit body before any differentiation has taken place. The deeply stained elements are nutritive hyphae.

Fig. 26. × 30 diam. An older stage, which shows the differentiation of stem and pileus primordia.

Fig. 27. × 192 diam. An enlargement of a fruit body, a little older than that of fig. 26, in which the primordium of the hymenophore is developing.

Figs. 28 and 29. × 30 diam. A median and tangential section of a fruit body just beginning to form gill salients. The palisade layer has not yet developed an even surface.

Figs. 30–34. × 30 diam. A series of sections showing young gill salients.

Figs. 35–39. × 30 diam. A series of sections of an older fruit body, showing the gills.

Figs. 40–42. × 30 diam. A series of sections from a still older fruit body.

Fig. 43. × 209 diam. A portion of the surface of the pileus of the fruit body from which figs. 40–42 were taken.

Fig. 44. × 209 diam. An enlargement of the section shown in fig. 28, from a fruit body which is forming gill salients before a definite even palisade layer is formed.

Fig. 45. × 209 diam. An enlargement of the edge of a gill from the fruit body of fig. 41. An even palisade layer is now formed.

Fig. 46. × 566 diam. A further enlargement of the preceding.

Fig. 47. × 192 diam. An enlargement of the gill salients of the section shown in fig. 32.

Plate III

Hygrophorus nitidus (figs. 48–66)

Fig. 48. × 31 diam. An early stage in which the pileus primordium is beginning to differentiate from that of the stem by the epinastic growth of the hyphae.

Fig. 49. × 31 diam. A slightly older stage in which stem, pileus and hymenophore fundaments are distinguishable.

Figs. 50 and 51. × 31 diam. An older stage, showing the primordium of the hymenophore further developed.

Fig. 52. × 210 diam. A higher magnification of the section of fig. 50 showing the hymenophore primordium.

Figs. 53–58. × 31 diam. A series of sections from a fruit body in which the gill salients are just beginning to form. The subadjacent tissue has become very loose.

Figs. 59–63. × 31 diam. An older fruit body, showing the development of the gills. Notice the very loose character of the subadjacent layer and the very irregular gill surface. The deeply stained hyphae on the surface of the pileus make up the slime-producing layer.

FIGS. 64 AND 65. × 12 diam. Median and tangential sections of a more mature fruit body. The plant is becoming umbilicate and the gills triangular in shape.

FIG. 66. × 210 diam. An enlargement of the section from which fig. 61 was taken, showing the uneven surface of the gills.

PLATE IV

Hygrophorus borealis (figs. 67–81)

FIG. 67. × 28 diam. Upper part of a young, oval, homogeneous fruit body.

FIGS. 68 AND 69. × 28 diam. Later stages. The buttons become very elongate.

FIGS. 70 AND 71. × 28 diam. The primordia of stipe, pileus and hymenophore have become differentiated.

FIG. 72. × 190 diam. An enlarged photograph of the margin of the pileus of the section of fig. 70.

FIGS. 73 AND 74. × 28 diam. Sections from a fruit body, showing the palisade layer.

FIG. 75. × 190 diam. The palisade layer of fig. 74 enlarged.

FIGS. 76–80. × 28 diam. A fruit body showing well-developed gill salients. The light area, which extends into the gills and between them in the pileus region subadjacent to the hymenophore, is the region which has given rise by branching to the elements of the hymenophore.

FIG. 81. × 28 diam. A nearly median section, with a portion of the gill attached to the stem.

PLATE V.

Entoloma flavifolium (figs. 82–100)

FIG. 82. × 35 diam. Young button in which the pileus primordium is beginning to develop.

FIGS. 83 AND 84. × 30 diam. A slightly older fruit body in which pileus, stem and hymenophore primordia have developed.

FIGS. 85 AND 86. × 28 diam. The palisade layer is distinguishable, although it does not take the stain readily.

FIGS. 87–90. × 28 diam. Serial sections from a fruit body in which the young gill salients are beginning to form.

FIGS. 91–95. × 28 diam. A series of sections from a more mature fruit body. Young basidia over the surface of the gills take the stain very readily.

FIGS. 96 AND 97. × 9 diam. Median and tangential sections of a nearly mature fruit body.

FIG. 98. × 160 diam. An enlargement of a section of a fruit body in about the stage of fig. 85, showing the palisade layer.

FIG. 99. × 191 diam. An enlargement of the section of fig. 89, showing very young gill ridges. Certain elements in the palisade layer are beginning to take the stain very readily.

FIG. 100. × 191 diam. An enlargement of the section of fig. 94, showing young basidia developing over the surface of the gills.

PLATE VI

Entoloma grayanum (figs. 101–121)

FIG. 101. × 33 diam. Young fruit body in which the pileus primordium is beginning to differentiate from that of the stem.

FIG. 102. × 33 diam. A slightly older stage.

FIG. 103. × 31 diam. Pileus and stem primordia have developed and the hymenophore fundament is commencing to form in the furrow between them.

FIGS. 104 AND 105. × 31 diam. Median and tangential sections of a fruit body showing the primordium better developed.

FIGS. 106–109. × 31 diam. A series of sections, showing the palisade layer, just previous to the formation of the gill salients.

FIG. 110. × 518 diam. Enlargement of the section of fig. 108, showing the palisade layer.

FIGS. 111–115. × 30 diam. A series of sections, forming young gill ridges.

FIGS. 116–120. × 30 diam. Slightly older stage, showing the same. The black dots appearing on the margin in fig. 119 are young basidia.

FIG. 121. × 191 diam. An enlargement of the gill salients of the section of fig. 119. The nuclei of the young basidia are very prominent.

PLATE VII

Entoloma cuspidatum (figs. 122–139)

FIG. 122. × 32 diam. Young undifferentiated fruit body, still attached to a rhizomorph.

FIG. 123. × 32 diam. The fruit body by growth at the apex is beginning to differentiate into the pileus primordium.

FIGS. 124 AND 125. × 32 diam. Older stages showing the pileus and stipe primordia.

FIGS. 126 AND 127. × 32 diam. Median and tangential sections of a fruit body, in which the primordium of the hymenophore has made its appearance.

FIGS. 128 AND 129. × 32 diam. Median and tangential sections from a fruit body, which has developed the palisade layer.

FIGS. 130–134. × 32 diam. A series of sections, showing the development of young gill salients.

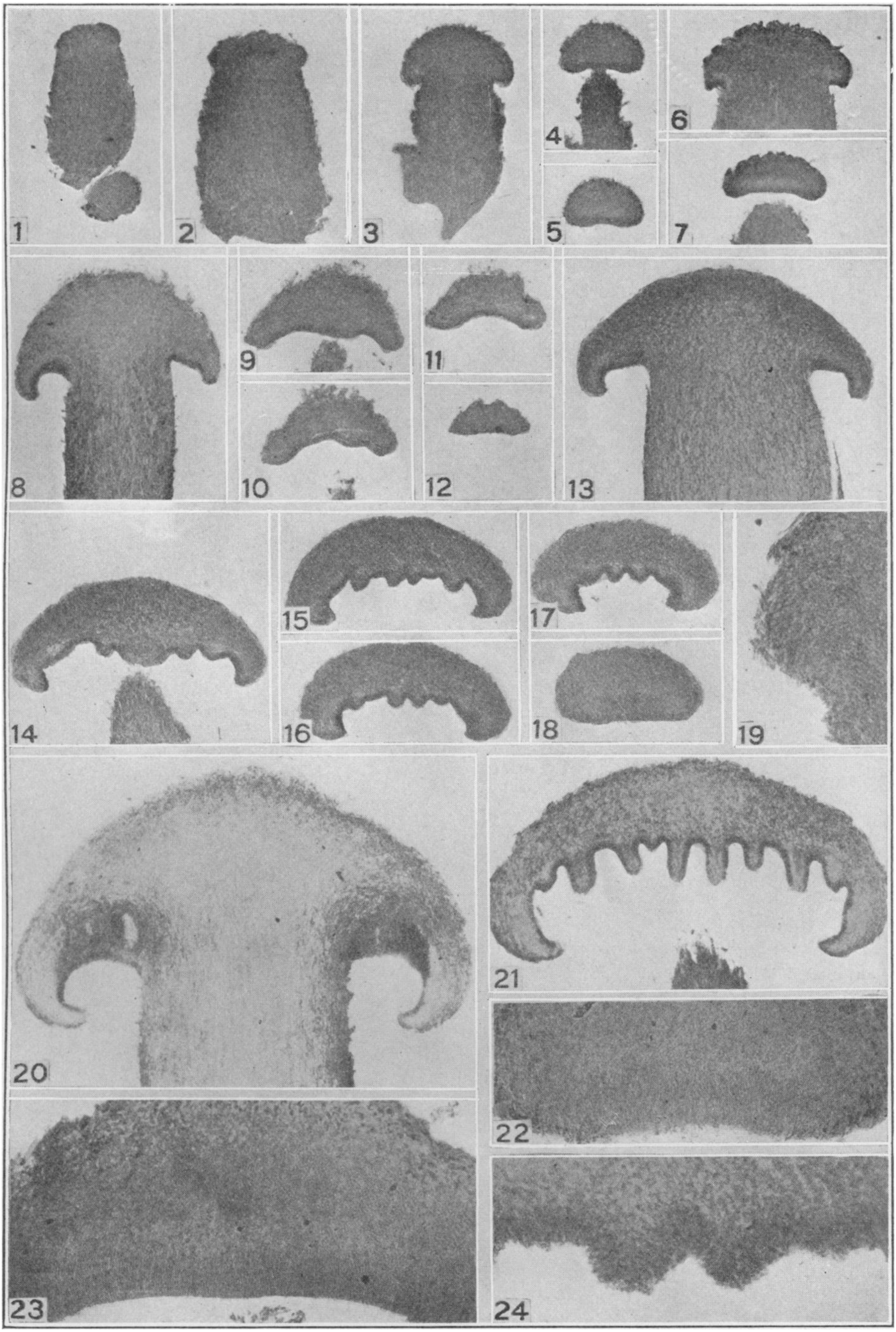
FIG. 135. × 29 diam. Median section of a nearly mature fruit body.

FIG. 136. × 213 diam. Section of the gill of a mature plant, showing the cubical basidiospores.

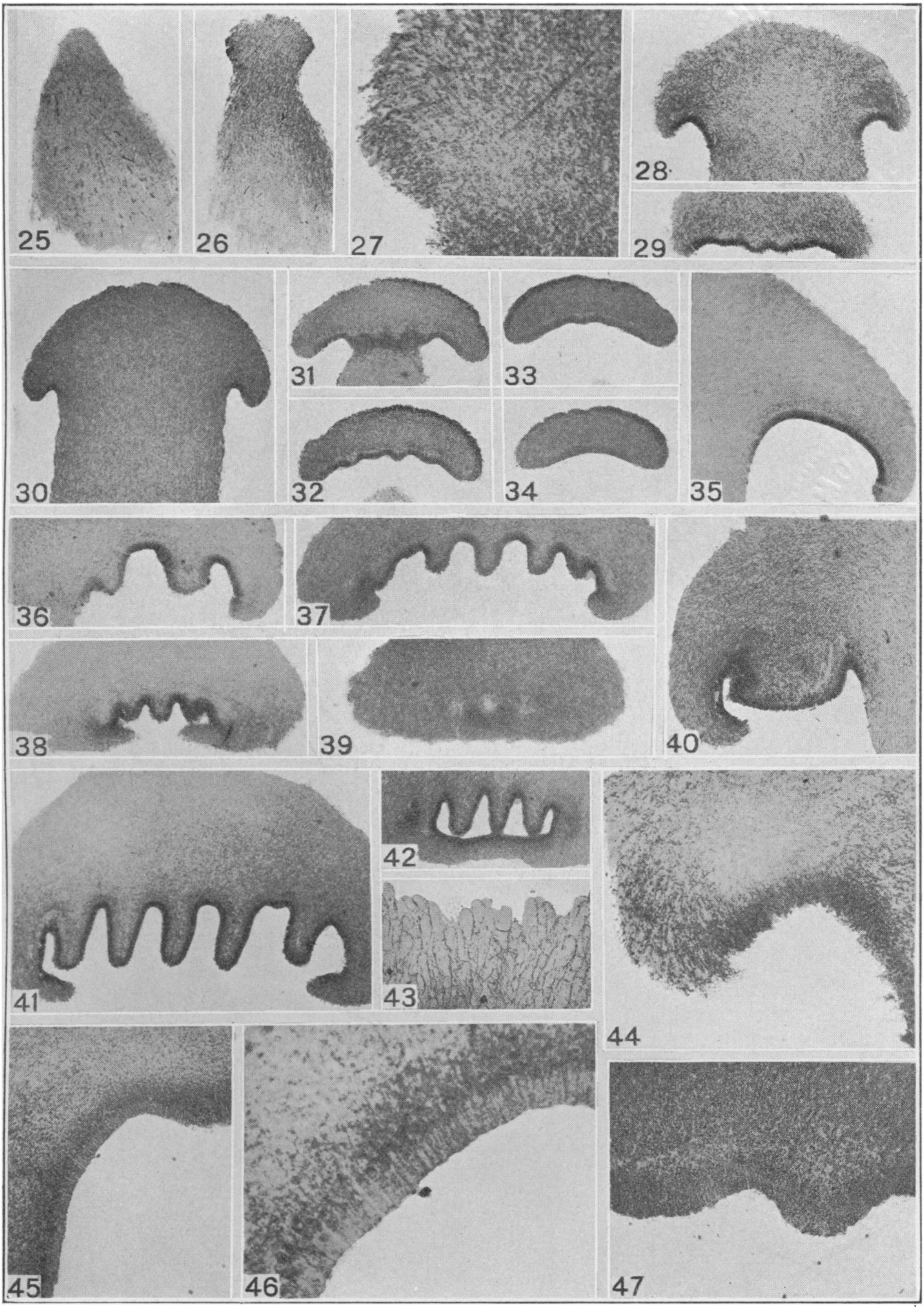
FIG. 137. × 213 diam. An enlargement of the section of fig. 128, showing the palisade layer.

FIG. 138. × 213 diam. An enlargement of the section of fig. 126, showing the hymenophore in a primordial state.

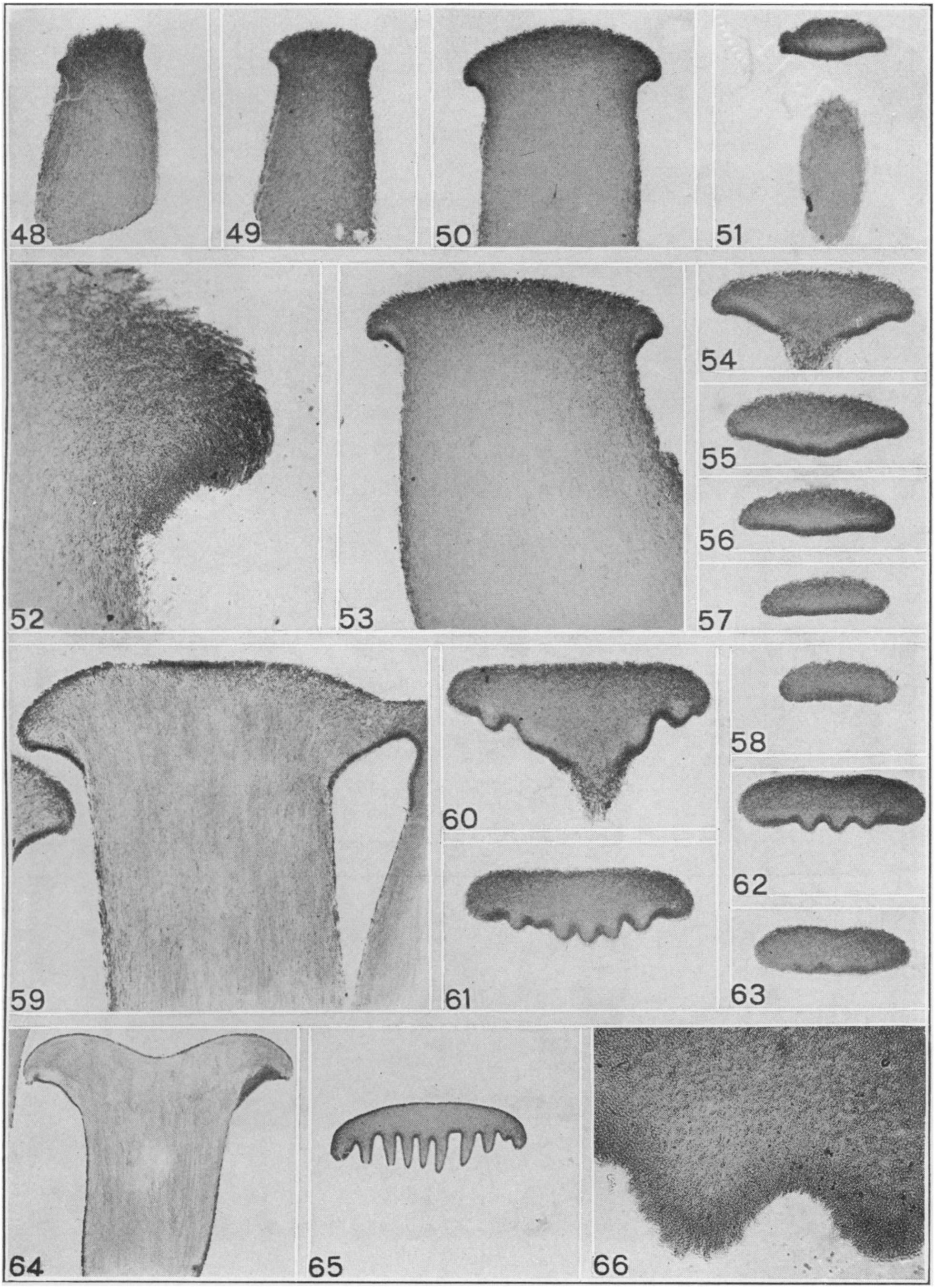
FIG. 139. × 213 diam. An enlargement of the section of fig. 134, showing the young gills.



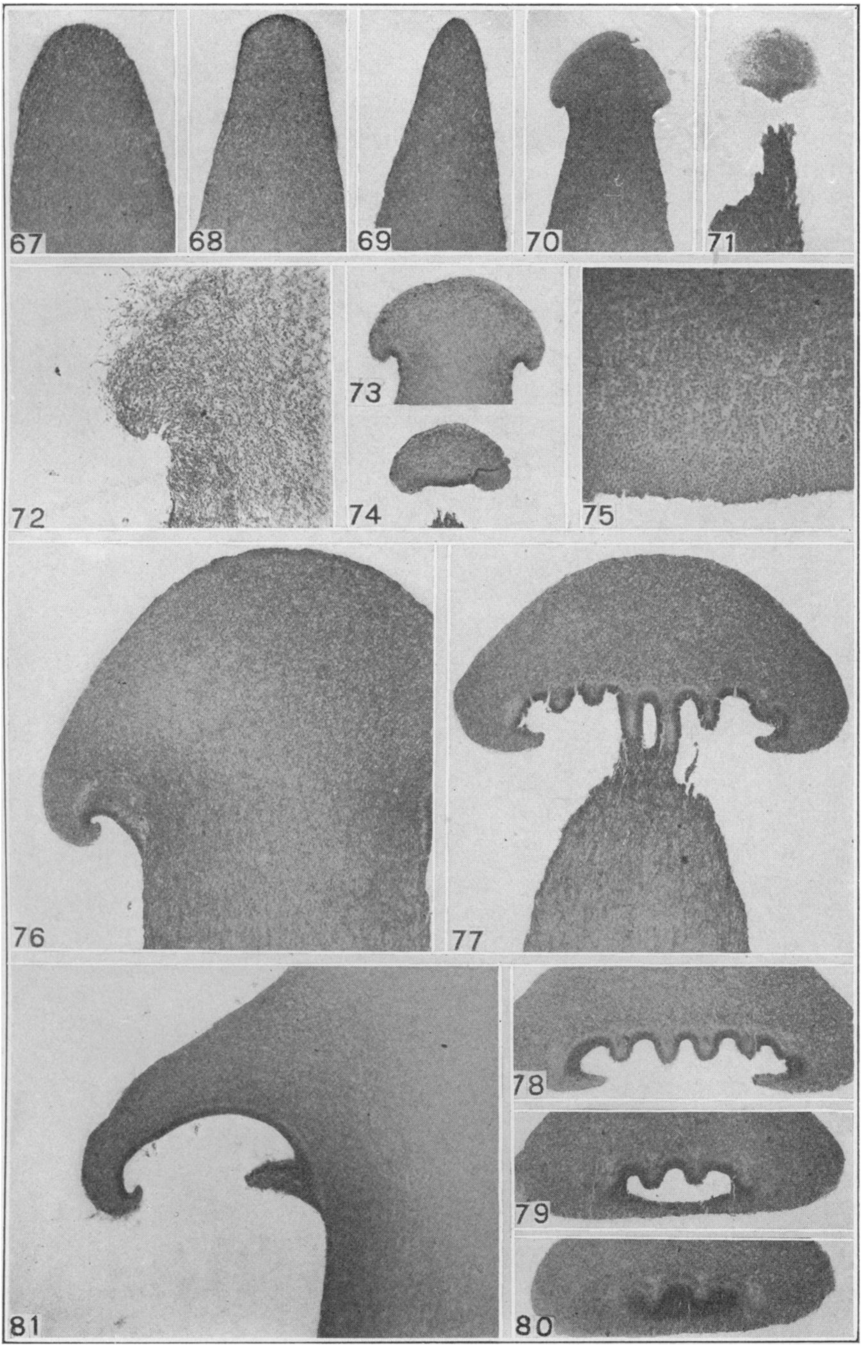
DOUGLAS: MYCENA SUBALCALINA.



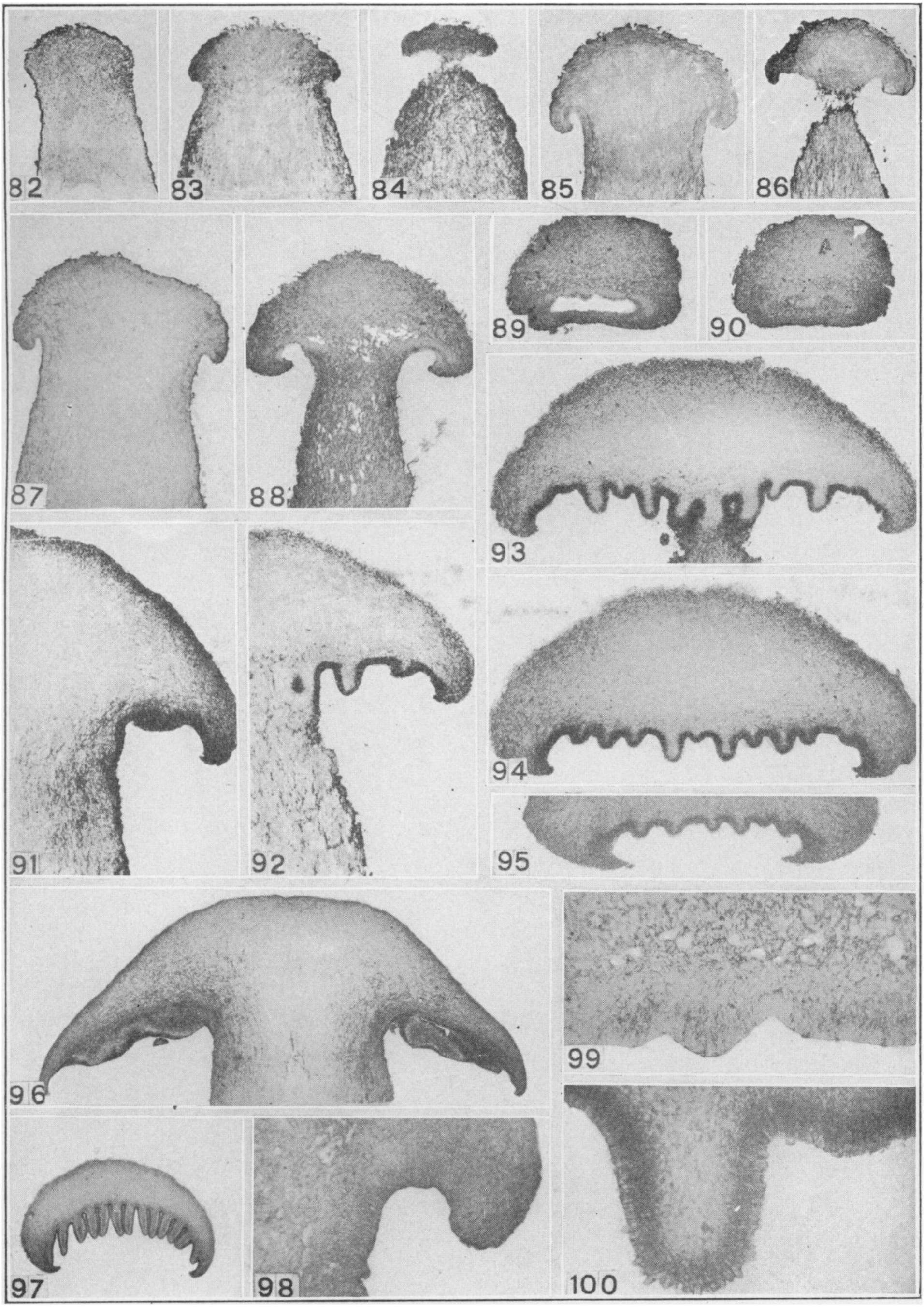
DOUGLAS: HYGROPHORUS MINIATUS.



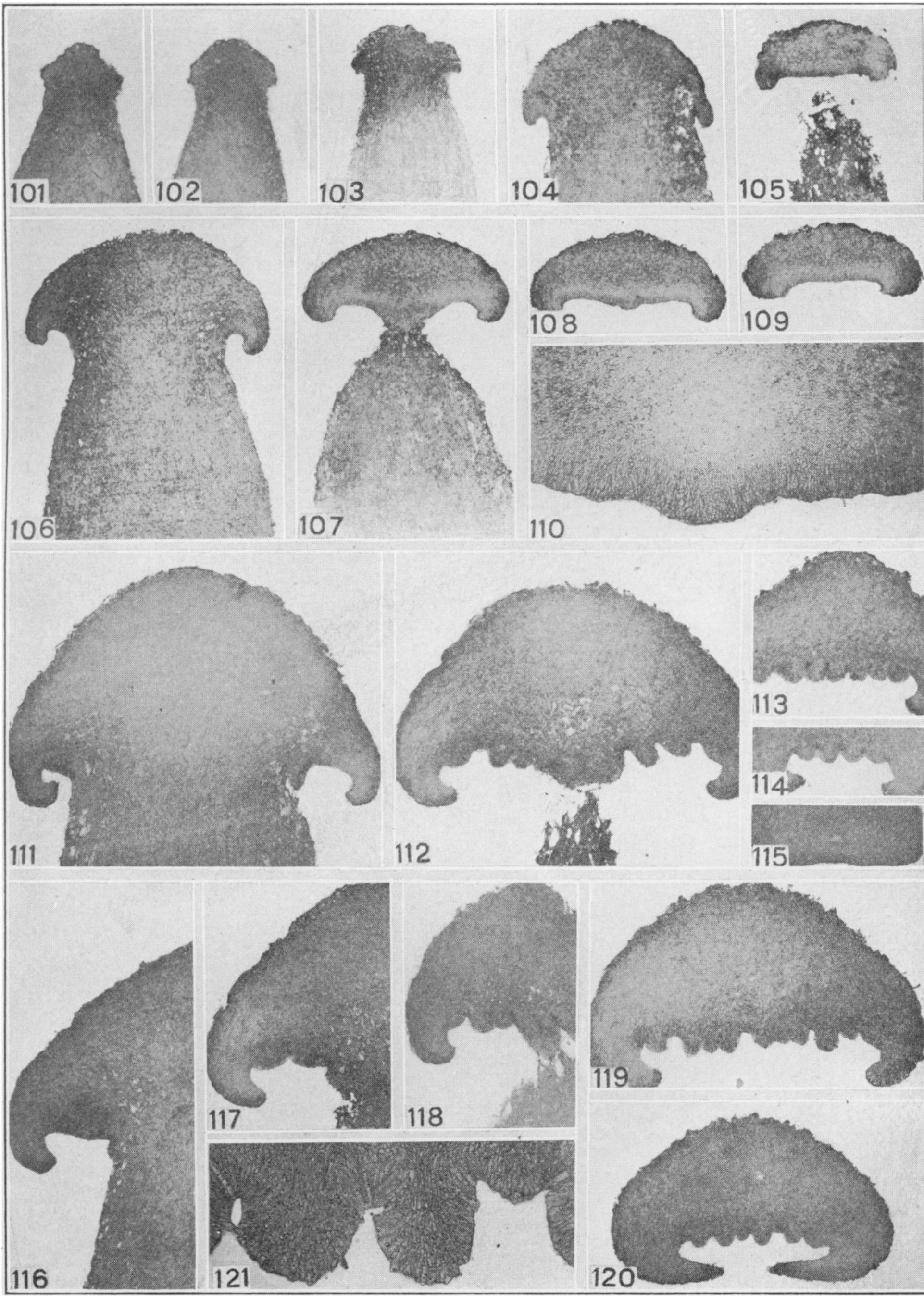
DOUGLAS: HYGROPHORUS NITIDUS.



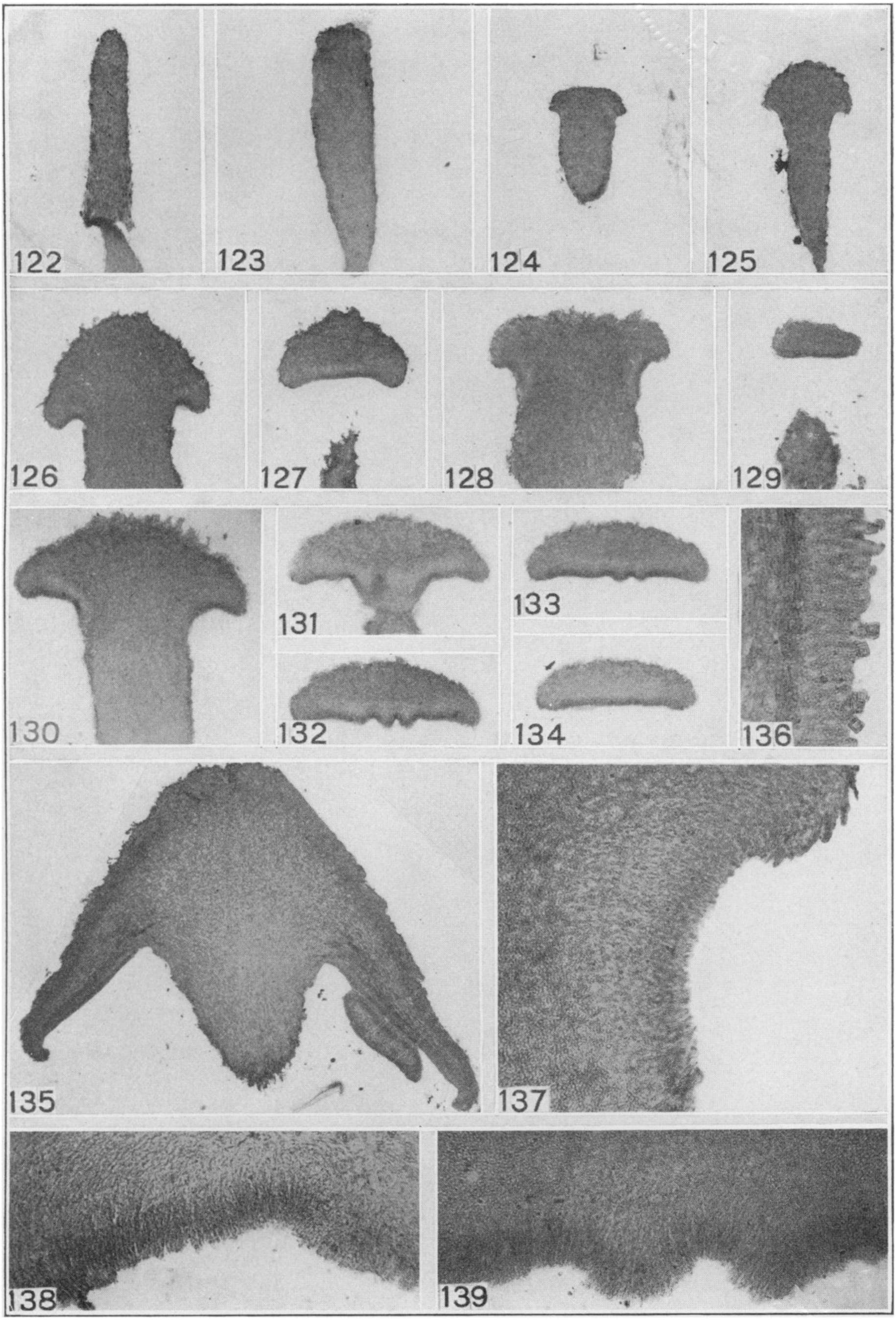
DOUGLAS: HYGROPHORUS BOREALIS.



DOUGLAS: ENTOLOMA FLAVIFOLIUM.



DOUGLAS: ENTOLOMA GRAYANUM.



DOUGLAS: ENTOLOMA CUSPIDATUM.